INTERLEUKIN-8 AND MONOCYTE CHEMOTACTIC ACTIVATING FACTOR RESPONSES TO CARDIOPULMONARY BYPASS

Cardiac operations with cardiopulmonary bypass cause a systemic inflammatory response. Neutrophils and monocytes-macrophages play an important role in triggering the initiation of the inflammatory response. Recently, some kinds of cytokines that are powerful leukocyte chemotactic factors have been characterized concerning an inflammatory response: interleukin-8 has a potent chemoattractant activity for neutrophils, and monocyte chemoattractant factor has monocyte-macrophage chemotactic activity. To investigate the possible roles of the cytokines in the inflammatory response in cardiopulmonary bypass, 12 adult patients undergoing cardiopulmonary bypass were studied for measurement of interleukin-8 and monocyte chemoattractant factor. Systemic blood was collected before cardiopulmonary bypass, at the end of cardiopulmonary bypass, and at 3, 12, 24, and 48 hours after cardiopulmonary bypass from the patients' radial arteries. Significant increases in levels of interleukin-8 and monocyte chemoattractant factor were detected with a peak level at 3 hours after bypass compared with levels before cardiopulmonary bypass ($p < 0.05$). This study demonstrated that interleukin-8 and monocyte chemoattractant factor are released into the circulation after adult hypothermic cardiopulmonary bypass and reach a maximum level 3 hours after bypass. (J THORAC CARDIOVASC SURG 1995;110:99-102)

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Cardiac operations with cardiopulmonary bypass (CPB) cause a systemic inflammatory response.[1-3] A degree of tissue injury and associated organ dysfunction, particularly in the heart and lungs, is usual after CPB.[3-5] This is thought to be a result principally of a combination of inflammation induced by (1) exposure of blood to an artificial surface[1, 3, 4, 6] and (2) reperfusion injury on discontinuation of bypass.[3, 7, 8] Recently, a group of cytokines with chemotactic and activating effects on leukocytes (so-called chemokines) has been described: interleukin-8 (IL-8) has potent chemoattractant activity for neutrophils,[9, 10] and monocyte chemoattractant factor (MCAF) has monocyte-macrophage chemotactic activity.[11, 12] To our knowledge, however, IL-8 has been reported previously in human beings during CPB by only a few researchers,[13, 14] and MCAF has not been reported during CPB.

On this basis, we performed a prospective self-controlled study to examine the circulating concentrations of IL-8 and MCAF before, during, and after operations with the use of CPB.

Patients and methods

Twelve adult patients (4 men, 8 women) undergoing elective cardiac and aortic operation with CPB at our institution comprised the study population. Patients' ages ranged from 48 to 75 years (mean 62.9 ± 9.9 years). All CPB procedures were done at moderate hypothermia (28° C) with cold blood cardioplegia. The duration of cardioplegic arrest varied from 29 to 168 minutes (mean 95.3 ± 12.0 minutes) and CPB times ranged from 89 to 234 minutes (mean 155.8 ± 12.0 minutes). The types of operations done were aortic valve replacement in five patients, mitral valve replacement in three patients, ascending aorta replacement in three patients, and ventricular septal perforation repair in one patient. All received a similar balanced anesthetic, including sufentanyl citrate,

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Table I. *Cytokine levels in serum (in picograms per milliliter)*

	pre-CPB	*end-CPB*	*3 hr*	*12 hr*	*24 hr*	*48 hr*
IL-8	2.3 ± 0.9	8.0 ± 2.0	187.3 ± 100.9*	23.6 ± 4.4	11.9 ± 2.9	2.8 ± 1.0
MCAF	97.1 ± 20.8	193.0 ± 32.2	2519.2 ± 940.0*	470.8 ± 144.4	153.8 ± 24.2	128.8 ± 25.1

Values are mean plus or minus the standard error of the mean. Each mean represents values from 12 patients.
End-CPB, At the end of CPB; *hr,* hours after CPB.
*$p < 0.05$ versus pre-CPB.

inhaled isoflurane, and a neuromuscular blocking agent. Steroids were not used in any of the patients.

Arterial blood samples were drawn from the patients' radial arterial lines as follows: before CPB, at the end of CPB, and 3, 12, 24, and 48 hours after CPB.

Serum was separated immediately by centrifugation and stored at −20° C until use. Serum concentration was measured by enzyme-linked immunosorbent assay kits (TORAY Inc., Shiga, Japan), which were previously developed.[15, 16] The assay was done in duplicate for each sample.

Cytokine levels at various occasions were compared with pre-CPB values by repeated-measures analysis of variance followed by Fisher's protected least significant difference with analysis of variance. Results are expressed as mean plus or minus the standard error of the mean, and $p < 0.05$ is considered to be significant.

Results

All patients had an uncomplicated recuperation and left the hospital uneventfully.

Table I presents pooled data for all patients showing changes in plasma concentrations of IL-8 and MCAF as a function of time. These data are represented graphically in Figs. 1 and 2. The influence of CPB on cytokine levels is shown by a significant increased in levels of both IL-8 and MCAF 3 hours after CPB compared with pre-CPB levels ($p < 0.05$). After the peak value was reached, concentrations fell in postoperative specimens. Although these remained detectable 24 hours later, the concentrations returned to baseline levels after 48 hours.

Discussion

Previous reports have indicated that infiltrating leukocytes play an important role in inflammatory response during surgical intervention, extracorporeal circulation, reperfusion injury, and infection. Recently, IL-8 and MCAF were characterized as potent leukocyte chemotactic and activating factors. IL-8 is produced by a variety of cell types including endothelial cells, monocytes, and T-lymphocytes. IL-8 is believed to have a key role in the accumulation of neutrophils in inflamed tissue.[9, 10] It upregulates complement receptor-1[17] and leukocyte adhesion molecule Mac-1 (CD11b/18)[18] and stimulates neutrophil adhesion to endothelial cells, as well as exerting potent chemoattractant activity for neutrophils,[9, 10] basophils, and T-lymphocytes.[19, 20] Although MCAF is produced by a variety of cells including endothelial cells, fibroblasts, and vascular smooth muscle cells, it activates and attracts monocytes. Also, IL-1 and tumor necrosis factor have been shown to induce IL-8 and MCAF gene expression at the transcriptional level.[11, 21]

Our results indicated that a significant increase in IL-8 was detected with a peak level at 3 hours after bypass. Previously, some researchers reported IL-8 plays a role as a major mediator of the acute phase response to CPB. Finn and colleagues[13] reported that levels of IL-8 rose at the time of rewarming, toward the end of bypass, and peaked 1 to 3 hours thereafter in children.[13] Kalfin and colleagues[14] reported that a significant increase in IL-8 levels was detected in circulating leukocytes with a peak level at 24 hours after bypass, but they did not perform measurements within 24 hours after bypass. They stated it is likely that IL-8 would have increased dramatically in the early post-CPB phase.[14]

To our knowledge, MCAF has not been reported previously in the human patient during CPB. Our results show that MCAF was also released and appeared in the systemic circulation in the early post-CPB phase and that it reached a peak level at 3 hours after bypass similar to the finding for IL-8. Kappelmayer and colleagues[22] reported that tissue factor expression and procoagulant activity are induced by monocytes during extracorporeal circulation, which leads to the suggestion that this may contribute to the increased risk of thromboembolic events during the period of prolonged extracorporeal circulation. These data suggest that activation of monocytes and macrophages during CPB may also be important from the thrombogenic point of view.

During CPB, it is suspected that inflammatory responses are induced by not only exposure of blood to artificial surfaces, but also by reperfusion injury

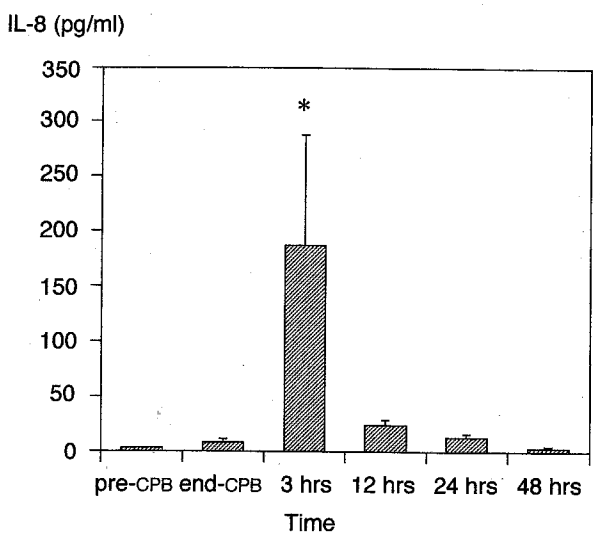

Fig. 1. Changes in circulating concentrations of IL-8 as function of time in patients undergoing CPB. Points of collection are just before initiation of CPB *(pre-CPB),* at end of CPB *(end CPB),* 3 hours after completion of CPB, 12 hours after CPB, 24 hours after CPB, and 48 hours after CPB. *Error bars* indicate standard error of mean. $*p < 0.05$ versus pre-CPB.

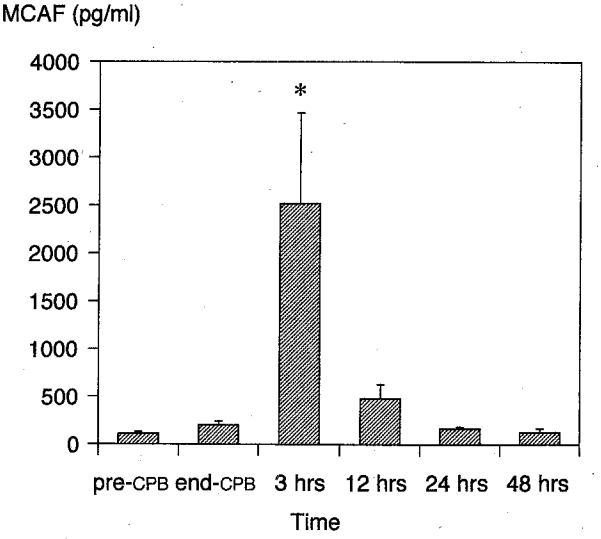

Fig. 2. Changes in circulating concentrations of MCAF as function of time in patients undergoing CPB. Points of collection are just before initiation of CPB *(pre-CPB),* at end of CPB *(end CPB),* 3 hours after completion of CPB, 12 hours after CPB, 24 hours after CPB, and 48 hours after CPB. *Error bars* indicate standard error of mean. $*p < 0.05$ versus pre-CPB.

on discontinuation of bypass.[1, 3, 4, 6-8] In experimental models of ischemia/reperfusion injury, it is considered that newly recruited neutrophils are capable of mediating extensive tissue damage.[23-25] Although it is presently unclear what role IL-8 plays in attracting neutrophils to reperfused tissue sites in vivo, some researchers have discussed the relationship between IL-8 and reperfusion injury.[26, 27] Clinically, Abe and colleagues[26] reported that the serum IL-8 concentration showed a transient rise during the early phase of acute myocardial infarction.

In summary, our preliminary results show that IL-8 and MCAF were released and appeared in the systemic circulation in the early post-CPB phase. These results suggest that these cytokines play a role as major mediators of the acute phase response to CPB and would provide sensitive parameters of inflammatory responses during CPB. Furthermore, if IL-8 and MCAF responses to CPB are important, there are several potential therapeutic possibilities. The ability of monoclonal antibodies against IL-8 and MCAF to inhibit postperfusion injury and inflammatory response may be, in part, secondary to a decrease in production of these chemokines, a subsequent reduction in the elimination of neutrophils, and a prevention of tissue damage after CPB.

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